

[RAPID COMMUNICATION]

**Biosynthesis of 17 α ,20 α -dihydroxy-4-pregnen-3-one
from 17 α -hydroxyprogesterone by Goldfish
(*Carassius auratus*) Spermatozoa**KIYOSHI ASAHINA¹, KATSUMI AIDA² and TEIZO HIGASHI¹¹College of Agriculture and Veterinary Medicine, Nihon University, Setagaya-ku,
Tokyo 154, and ²Department of Fisheries, Faculty of Agriculture,
The University of Tokyo, Bunkyo-ku, Tokyo 113, Japan

ABSTRACT—Goldfish (*Carassius auratus*) spermatozoa were incubated with 4-¹⁴C-labeled 17 α -hydroxyprogesterone at 22°C for 60 min. After extraction of the steroids from the incubation medium, they were separated by thin-layer chromatography (TLC). Radioactive steroids were localized on the TLC-plates by autoradiography. The major metabolite showed an *R_f* value lower than that of 17 α ,20 β -dihydroxy-4-pregnen-3-one (17 α ,20 β -P) but co-migrated with 17 α ,20 α -P, the isomer of 17 α ,20 β -P. The TLC behaviour of the metabolite was identical with authentic 17 α ,20 α -P after both acetylation and oxidation. The identity of the metabolite with 17 α ,20 α -P was finally confirmed by repeated crystallization to constant specific activity. These results indicate that goldfish sperm cells show 20 α -hydroxysteroid dehydrogenase activity.

INTRODUCTION

The mature sperm cells of the rainbow trout (*Oncorhynchus mykiss*) produce very high levels of the steroid 17 α ,20 β -dihydroxy-4-pregnen-3-one (17 α ,20 β -P) when incubated with 17 α -hydroxyprogesterone (17 α -P) *in vitro* [11, 14]. In contrast, it was recently found that spermatozoa of the common carp (*Cyprinus carpio*) produce 17 α ,20 α -dihydroxy-4-pregnen-3-one (17 α ,20 α -P), the isomer of 17 α ,20 β -P, when incubated with 17 α -P [1]. In the present study we investigated which 17 α -P metabolites were formed by spermatozoa of another cyprinid fish, the goldfish (*Caras-*

sius auratus).

MATERIALS AND METHODS*Chemicals*

4-¹⁴C-Labeled 17 α -hydroxyprogesterone (*17 α -P; specific activity, 1.85 GBq/mmol) was obtained from Du Pont (UK). All organic solvents were of special grade and were obtained from the Tokyo Kasei Chemical Co. (Tokyo, Japan). 17 α ,20 α -Dihydroxy-4-pregnen-3-one (17 α ,20 α -P), and other non-radioactive steroids and chemicals were obtained from the Sigma Chemical Company (St. Louis, U.S.A.).

Fish

Male goldfish were kept with females in 5×0.8×0.6 m tanks under natural temperature and photoperiod conditions at the University of Tokyo; they were fed with commercial trout pellets. In April, 1991, five spermiating males were selected from the tank. Milt samples were collected in glass tubes from their genital pores by applying gentle pressure to the abdomen. Milt from all five fish was pooled and transported on ice to the department of fisheries, faculty of agriculture and veterinary medicine, Nihon University.

Incubation

Sperm cells were washed twice with carp Ringer

and collected with centrifugation at 2000 rpm for 15 min. The incubation was carried out in 5 ml of carp culture medium [8] containing 240 mM NADPH, ^{14}C - $17\alpha\text{-P}$ (1×10^5 cpm), and approximately 100 mg wet weight of washed spermatozoa. They were incubated in a 50 ml round-bottomed flask for 1 hr at 22°C. The incubation was stopped by adding 15 ml dichloromethane and shaking vigorously for one minute.

Extraction and TLC

The procedures were the same as described previously [2]. In short, the incubation medium containing the spermatozoa was extracted twice with dichloromethane. The extracts were pooled and dried with anhydrous Na_2SO_4 . After evaporation of the solvent, the residues were subjected to thin-layer chromatography (TLC) with six unlabeled steroids for standards; progesterone, $17\alpha\text{-P}$, androstenedione, testosterone, $17\alpha,20\beta\text{-P}$ and $17\alpha,20\alpha\text{-P}$ in a benzene:acetone (4:1, v/v) solvent system. After development, the standard steroids were detected under UV light and radioactive spots on the chromatograms were detected autoradiographically by exposing the TLC plate to a sheet of medical X-ray film for one week. Each spot was then separately scraped off the plate. Radioactive compounds were eluted from the silica gel with a mixture of chloroform and ethanol (1:1, v/v), and aliquots were used to determine the radioactivity by liquid scintillation counting. The metabolites were identified on the basis of the following criteria:

(1) Isopolarity with authentic steroids on TLC developed in two systems of the following solvent mixture: benzene:acetone, 4:1 (v/v) and dichloromethane:diethyl ether, 5:2 (v/v).

(2) Identical chemical behaviour of the radioactive metabolites with the authentic preparations after acetylation [15] and oxidation [10].

(3) Constant specific radioactivity of crystals after repeated crystallization of the radioactive metabolite with the corresponding authentic preparation.

RESULTS

In the initial thin-layer chromatogram de-

veloped in a benzene:acetone (4:1, v/v) system, only one clear band ($R_f = 0.24$) corresponding to authentic $17\alpha,20\alpha\text{-P}$ was detected, in addition to the unchanged radioactive substrate, ^{14}C - $17\alpha\text{-P}$. Following scintillation counting it was found that the conversion of radioactivity to $17\alpha,20\alpha\text{-P}$ was approximately 34 times greater than that obtained from the area of authentic $17\alpha,20\beta\text{-P}$ (Table 1). The mobility of the metabolite was also identical with authentic $17\alpha,20\alpha\text{-P}$ on TLC using dichloromethane:diethyl ether (5:2, v/v) as solvent system. Furthermore, it behaved identically with authentic $17\alpha,20\alpha\text{-P}$ after both acetylation and oxidation. The identity of the metabolite was finally confirmed by repeated crystallization to constant specific activity with authentic $17\alpha,20\alpha\text{-P}$ (Table 2).

TABLE 1. *In vitro* conversion of 17α -hydroxyprogesterone by goldfish (*Carassius auratus*) spermatozoa

Metabolite	Yield
Unchanged substrate (17α -hydroxyprogesterone)	753.1*
$17\alpha,20\alpha$ -dihydroxy-4-pregnen-3-one	64.6
$17\alpha,20\beta$ -dihydroxy-4-pregnen-3-one**	1.9

* pmol of steroid/100 mg tissue/60 min

** Tentatively identified.

TABLE 2. Recrystallization of the radioactive metabolite with the authentic preparation of $17\alpha,20\alpha$ -dihydroxy-4-pregnen-3-one for identification

	Specific radio activity (cpm/mg)
Before recrystallization	371
After recrystallization	
1st (dichloromethane- <i>n</i> -hexane)	333
2nd (dichloromethane- <i>n</i> -heptane)	361
3rd (chloroform- <i>n</i> -hexane)	350

DISCUSSION

Our results indicate that mature sperm cells of the goldfish contain the enzyme 20α -hydroxysteroid dehydrogenase ($20\alpha\text{-HSD}$) and produce

significant levels of $17\alpha,20\alpha$ -P when incubated with 17α -P for one hour *in vitro*. In addition, low levels of radioactivity co-migrated with authentic androstenedione. However, there was too little radioactivity for further analysis.

$17\alpha,20\alpha$ -P is reported to be synthesized *in vitro* in the ovarian tissues of a marine teleost, the dab [5]. In the dab, high levels of this steroid were detected after hCG treatment of both female [4] and male fish [3]. Because the 20α -HSD activity was suggested to reside in dab spermatozoa [5], the sperm cells may be responsible for the synthesis of plasma $17\alpha,20\alpha$ -P. Recently, $17\alpha,20\alpha$ -P was also shown to be synthesized by goldfish ovary *in vitro* [6].

We did not detect a clear 20β -HSD activity in goldfish spermatozoa after 60 min of incubation. However, increased plasma levels of $17\alpha,20\beta$ -P were detected with radioimmunoassay in male goldfish in relation to spawning behaviour [7] and after the administration of GTH [12]. Since a low 20β -HSD activity was detectable after a long term (18 hr) incubation of common carp spermatozoa [1], it cannot be ruled out that goldfish sperm cells also show low 20β -HSD activity. This, however, was almost undetectable after our short term incubation.

$17\alpha,20\beta$ -P has been reported to be effective by inducing spermiation in amago-salmon and goldfish [13], and the acquisition of sperm motility in salmonid fish [9]. From our results, however, it is reasonable to hypothesize that $17\alpha,20\alpha$ -P also has some role in regulating spermiation in the goldfish. Recently we have found that during spawning the $17\alpha,20\alpha$ -P plasma levels increase to almost the same levels as those of $17\alpha,20\beta$ -P in both male and female goldfish (Asahina *et al.*, unpublished results). The reason(s) for the discre-

pancy between the *in vitro* production and the plasma levels of these two steroids is currently under investigation.

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